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(54) Title: INSULIN POTENTIATING PEPTIDES

(57) Abstract: This invention relates to compounds which have the ability to potentiate the physiological activity of insulin, and in particular to small peptide compounds. The compounds are useful in the treatment of conditions related to insulin resistance, such as non-insulin dependent diabetes mellitus (NIDDM) and obesity. The invention provides a peptide or peptidomimetic compound which has the ability to potentiate one or more of the physiological activities of insulin, in which the peptide comprises the sequence: W-X-Y-Z where W is a basic amino acid, such as lysine, arginine, homolysine, homoarginine or ornithine; X is a neutral aliphatic amino acid, in either the L- or the D-form, such as glycine, leucine, alanine, β-alanine or isoleucine, homoleucine, norleucine, homonorleucine, cyclohexylalanine, or homocyclohexylalanine; Y is an aromatic amino acid, such as phenylalanine or tyrosine; and Z is an amino acid or amino acid analogue which has a side chain having π or delocalised electrons, with the proviso that the peptide is not Arg-Gly-Phe-Phe, Arg-Gly-Ser-Arg-Leu-Phe-Phe-Asn-Tyr-Ala-Leu-Val, Arg-Leu-Phe-Asu-Asn-Ala, or Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala. Compositions and methods of treatment are also within the scope of the invention.

INSULIN POTENTIATING PEPTIDES

This invention relates to compounds which have the ability to potentiate the physiological activity of insulin, and in particular to small peptide compounds. The compounds are useful in the treatment of conditions related to insulin resistance, such as non-insulin dependent diabetes mellitus (NIDDM) and obesity.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Insulin resistance is a physiological state in which insulin induces a diminished response from target tissues. This resistance to insulin action is a major pathogenic factor associated with non-insulin-dependent diabetes mellitus (NIDDM) (Keen, 1994), obesity (Felber *et al*, 1993; Truglia *et al*, 1985), hypertension (Baba and Neugebauer, 1994), and coronary heart disease (CHD) (Zavaroni *et al*, 1989).

Type II diabetes (non-insulin dependent diabetes) is characterised by inadequate control over blood sugars with an elevated level of plasma insulin. The biochemical causes are known to vary between individuals, although a common element in the development of an insensitivity is the deficiency of the target organs to respond to plasma insulin. Subsequently the pancreas has increasing difficulty supplying the increasing amount of insulin required to achieve the optimal blood glucose levels, particularly after meals. The insulin-producing islet

cells of the pancreas ultimately suffer from excessive use and begin to fail, further limiting the amount of insulin which can be produced. At this stage the patient may become overtly type I diabetic, requiring insulin doses to maintain blood glucose.

Risk factors for type II diabetes include old age, obesity and inherited genetic factors. There does not appear to be a dominant biochemical defect which causes the underlying insulin insensitivity. In principle, insulin insensitivity may be caused by interference with insulin before binding with the insulin receptor, receptor defects, defects at any of many possible points in the intracellular signalling pathways, defects in the glucose transport channels which insulin upregulates, or any combination of these factors.

The standard initial step in therapy is modification of diet and lifestyle. If this fails, a range of pharmaceutical agents is available for treating the condition, such as sulphonylureas, biguanides and thiazolidinediones. Perhaps because the disease has no common biochemical cause, responses to the drugs differ between individuals, and the drugs have significant side- effects.

The insulin-potentiating effects of certain synthetic peptide amides corresponding to the C-terminal fragment of the B-chain of insulin have been demonstrated by ourselves and others (Ng *et al*, 1989; Weitzel *et al*, 1971). The insulin B-chain(INSB) fragment from amino acid residues 22-25, Arg-Gly-Phe-Phe, has been shown to be involved in binding of the insulin molecule to its receptor (Pullen *et al*, 1976). This fragment is referred to herein as INSB(22-25). De Meyts *et al* reported that the INSB(22-25) fragment interacted with the residues 83-94, Arg-Gly-Ser-Arg-Leu-Phe-Phe-Asn-Tyr-Ala-Leu-Val, of the α-subunit of the insulin receptor (De Meyts *et al*, 1990). The remarkable resemblance between these sequences in insulin and its receptor apparently facilitates insulin-receptor binding by means of a Phe^{B25}-Phe^{89} interaction, which is similar to the Phe^{B25}-Phe^{B25} interaction in insulin dimerization.

In the early 1980s, similar insulin-potentiating effects were also shown both *in vitro* and *in vivo* with peptide amides from the amino-terminus of human growth hormone (hGH) (Ng *et al*, 1980). It was found that the peptide required an α-aminosuccinimide (Asu) modification in the residue Asp^{11} for biological activity (Robson *et al*, 1990). Asu^{11}-hGH(6-13) peptide, Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala, was shown to improve glucose tolerance in the insulin-resistant Zucker fatty (fa/fa) rats, as demonstrated by the glucose clamp technique (Lim *et al*, 1995). The amino acid sequence 8-11 of hGH, Arg-Leu-Phe-Asu-Asn-Ala, incorporating the Asn modification, elicits an insulin-potentiating effect. The four resides at the amino terminus of this peptide appear to be homologous to the corresponding sequence of the insulin tetrapeptide INSB(22-25). Conformational analysis of this peptide using NMR and molecular modelling suggested that a structural constraint, a Type II'β turn, was introduced by Asu (Ede *et al*, 1994).

It is known that peptides containing the minimal sequence hGH(6-13) are hypoglycaemic, and this sequence appears to account for the hypoglycaemic actions of intact hGH(1-191). The *in vitro* effects of hGH(6-13) include:

(a) facilitation of insulin binding to membrane receptors;

(b) acceleration of glucose transport in isolated cells;

(c) activation of intracellular enzymes for glucose and glycogen metabolism;

(d) augmentation of glucose oxidation in muscle, adipose tissue and liver; and

(e) enhancement of glucose-induced release of insulin from pancreatic islets.

The *in vivo* effects of hGH(6-13) include an increase of glucose disposal in glucose tolerance tests without causing excessive hypoglycaemia, and enhanced tissue sensitivity to the action of insulin.

The similar insulin-potentiating actions of peptide fragments from insulin, insulin receptor, and hGH may be due to

a common functional motif. The present study was therefore undertaken in order to identify the insulin-potentiating motif, based on the sequence structures of insulin, insulin receptor and hGH, with the objective of developing novel drugs in the treatment of NIDDM and their effects on obesity.

Insulin-potentiating effects were demonstrated both *in vitro* and *in vivo* with a series of peptide amide analogues corresponding to the amino acid sequence 22-25 of the B-chain of insulin, residues 86-89 of the α-subunit of insulin receptor, and the N-terminal region of human growth hormone. Structure-function studies suggest that the biological action may be intrinsic to a four-residue motif with a basic amino acid in position 1, a neutral aliphatic amino acid in position 2, an aromatic amino acid in position 3, and an amino acid with a side-chain having π or non-binding electrons in position 4. This molecular motif provides a new direction for the construction of novel therapeutic agents for the treatment of insulin-resistance related diseases such as non-insulin dependent diabetes mellitus (NIDDM) or obesity.

SUMMARY OF THE INVENTION

According to a first aspect, the invention provides a peptide which has the ability to potentiate one or more of the physiological activities of insulin, in which the peptide comprises the sequence:

W-X-Y-Z

where W is a basic amino acid, such as lysine, arginine, homolysine, homoarginine or ornithine;

X is a neutral aliphatic amino acid, in either the L- or the D-form, such as glycine, leucine, alanine, β- alanine or isoleucine, homoleucine, norleucine, homonorleucine, cyclohexylalanine, or homocyclohexylalanine;

Y is an aromatic amino acid, such as phenylalanine or tyrosine; and

Z is an amino acid or amino acid analogue which has a side chain having π or delocalised electrons,

with the proviso that the peptide is not Arg-Gly-Phe-Phe, Arg-Gly-Ser-Arg-Leu-Phe-Phe-Asn-Tyr-Ala-Leu-Val, Arg-Leu-Phe-Asu-Asn-Ala, or Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala.

Preferably the amino acid or amino acid analogue Z is one with a cyclic side chain, such as phenylalanine, tyrosine, tryptophan, α-amino succinimide, homophenylalanine or histidine.

It will be clearly understood that the sequence W-X-Y-Z is a minimum sequence, and may be extended at either the N- or C-terminal, provided that the ability to potentiate insulin activity is retained.

While the invention has been primarily exemplified in relation to peptides, it will also be understood that the peptide linkage between the residues may be replaced by a non-peptide bond provided that the ability to potentiate insulin activity is retained. The person skilled in the art will be aware of suitable such modifications.

Sequences encompassing conservative substitutions of amino acids are also within the scope of the invention, provided that the biological activity is retained.

It is to be clearly understood that the compounds of the invention include peptide amides and non-amides, and peptide analogues, including but not limited to the following:

1. Compounds in which one or more amino acids is replaced by its corresponding D-amino acid. The skilled person will be aware that retro-inverso amino acid sequences can be synthesised by standard methods; see for example Chorev and Goodman, 1993;

2. Peptidomimetic compounds, in which the peptide bond is replaced by a structure more resistant to metabolic degradation. See for example Olson *et al*, 1993; and

3. Compounds in which individual amino acids are replaced by analogous structures for example, *gem*-diaminoalkyl groups or alkylmalonyl groups, with or without modified termini or alkyl, acyl or amine substitutions to modify their charge.

The use of such alternative structures can provide significantly longer half-life in the body, since they are more resistant to breakdown under physiological conditions, or to improve bioavailability.

Methods for combinatorial synthesis of peptide analogues and for screening of peptides and peptide analogues are well known in the art (see for example Gallop *et al*, 1994; Hogan, 1997). It is particularly contemplated that the compounds of the invention are useful as templates for design and synthesis of compounds of improved activity, stability and bioavailability. Mimetics of amino acid side chains are known in the art. For example, mimetics of arginine side chains are disclosed in PCT/AU98/00490 (WO 99/00406) by The University of Queensland.

In a preferred embodiment of the invention, the peptide is selected from the group consisting of:

Arg-D-Ala-Phe-Phe	(SEQ ID NO. 3),
Arg-Leu-Phe-Phe	(SEQ ID NO. 4),
Arg-Leu-Phe-Asu-Asn-Ala	(SEQ ID NO. 6),
Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala	(SEQ ID NO. 7),
Leu-Ser-Lys-Leu-Phe-Asu-Asn-Ala	(SEQ ID NO. 8),
Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala	(SEQ ID NO. 10),
Arg-β-Ala-Phe-Phe	(SEQ ID NO. 18),
Arg-Gly-Tyr-Phe	(SEQ ID NO. 19),
Arg-D-Ala-Phe-Tyr	(SEQ ID NO. 22),
Arg-D-Ala-Phe-Tyr-me	(SEQ ID NO. 23), and
Arg-D-Ala-Tyr-Phe	(SEQ ID NO. 24).

More preferably the peptide is Arg-D-Ala-Phe-Phe-NH_2 (SEQ ID NO. 3)or Arg-D-Ala-Tyr-Phe-NH_2 (SEQ ID NO. 24).

In a second aspect, the invention provides a composition comprising a peptide according to the invention, together with a pharmaceutically-acceptable carrier.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton,

Pennsylvania, USA., and may be selected according to the desired route of administration.

In a third aspect, the invention provides a method of treatment of a pathological condition associated with insulin resistance, comprising the step of administering an effective amount of a peptide according to the invention to a subject in need of such treatment. Preferably the condition is non-insulin dependent diabetes mellitus or obesity. More preferably the condition is non-insulin-dependent diabetes mellitus.

In a fourth aspect, the invention provides a method of treatment of a pathological condition associated with insulin resistance, comprising the step of administering an effective amount of a compound which mimics the action of the binding region of INSB 22:25 on the insulin receptor to a subject in need of such treatment.

The dose and route of administration will depend on the nature of the condition to be treated, and the condition, previous treatment and general state of health of the subject to be treated, and will be at the discretion of the attending physician. However, in general it is contemplated that the dose will be in the range 0.1 to 100 mg/kg body weight, preferably 1 to 50 mg/kg body weight, more preferably 1 to 10 mg/kg body weight.

Although any desired route of administration may be used, including both enteral and parenteral routes such as oral administration or subcutaneous or intramuscular injection, preferably the peptide is administered orally or sublingually. One or more doses per day may be administered, preferably at meal times so as to reduce the peak post-prandial blood glucose level.

While the biological activity is demonstrated herein by measuring *in vitro* and *in vivo* insulin-potentiating effects, it will be clearly understood that primary screening of putative insulin-potentiating peptides may be achieved by any convenient method, preferably a high-throughput method of measuring binding to insulin receptor, using biosensor assays.

Suitable methods are well known in the art. It will be also understood that putative peptides and peptidometic compounds may readily be synthesised using automated high-throughput solid phase peptide synthesis.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the sensitivity of hemidiaphragm muscle tissue to the effect of insulin on glucose incorporation into glycogen. Mean ± SEM; data from 8 animals.

Figure 2 shows the effects of peptide 1 (Δ), peptide 2 (■), peptide 3 (▼), peptide 4 (▲), peptide 5 (□) and peptide 6 (●) (panel A) and peptide 7 (○) and peptide 8 (●), peptide 9 (▽), peptide 10 (Δ), peptide 11 (▲) (panel B) on the rate of glucose incorporation into glycogen in hemidiaphragm tissue by increasing concentrations of peptides, together with exogenous insulin (1 mU/ml). Tissues from the same rat were used for all groups. Mean ± SEM; data from 8 animals.

Figure 3 shows the effect of peptide 1 (Δ), peptide 2 (■), peptide 3 (▼), peptide 4 (▲), peptide 5 (□), and peptide 6 (●) on blood glucose levels of Zucker rats. Animals were given i.p. saline or peptide (10 μmol/kg body weight), and the reductions of blood glucose were determined. Basal blood glucose level of all animals were 6.2 ± 0.5 mmol/L before experimentation. * denotes that differences between the peptide treated and buffer control groups (○) are statistically significant ($p<0.05$) at the indicated time.

METHODS

Animals

Zucker fatty (fa/fa) female rats (440-470 g) of 30 weeks old and normal Wistar male rats (140-160 g) of 5 weeks old were used. The animals were fed *ad libitum* on rat pellets

(Clark King, Melbourne, Australia) with free access to water at all times, and housed in the departmental animal house.

Peptide synthesis

The peptide amide analogues discussed in Examples 1-6 were prepared by manual solid-phase synthesis, using the Fmoc strategy and Rink amide resin. The *in situ* coupling reaction was performed with diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazol (HOBt). After synthesis, the peptide was cleaved from the resin and side-chain protective groups were removed by treatment with Reagent K (King *et al*, 1990) for 1.5 hr, either at room temperature for peptides 1-4 or at 4ºC for peptides 5-11. Peptides were purified by reverse phase high performance liquid chromatography (RP-HPLC) using a preparative C18-column (21.2 mm x 25 cm, Supelco) and an acetonitrile gradient (0-50% in 50 min). The purity of peptides was at least 99%. The amino acid composition and the molecular weight determinations were determined either using a Waters Pico Tag system or by fast atom bombardment-mass spectrometry (FAB-MS).

In vitro measurements of glycogen synthesis in muscle

In vitro insulin-potentiating effects of the synthetic peptide analogues were assessed by measuring the rates of exogenous glucose incorporation into glycogen in rat hemidiaphragms (Lim *et al*, 1992). Hemidiaphragms from overnight-fasted Zucker fatty(fa/fa) female rats were dissected, and divided into segments of approximate 35-50 mg each. Tissues from the same rat were used for all groups. The tissue was incubated in 2 ml of Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing [^{14}C]glucose (5.5 mM, final specific activity 0.05 mCi/mmol) under an atmosphere of 95% O_2-5% CO_2 at 37ºC for 1.5 hr. After incubation, tissues were removed, washed with cold buffer and blotted. Tissues were digested, the muscle glycogen was precipitated and the ^{14}C-radioactivity was counted in a Wallac 1410 liquid scintillation counter. The biological activity of peptide analogues was measured as the

rate of mmol glucose incorporation into muscle glycogen/g tissue/hr.

The sensitivity of hemidiaphragm muscle tissue to insulin (0.1-100 mU/ml) on glycogen synthesis was first analyzed. The dose response curves for peptide analogues on the insulin-potentiating effect to glycogen synthesis were then measured using cumulative increasing concentrations (10^{-3} - 10 µmol/ml) of peptides in the presence of insulin (1 mU/ml). The biological activity of each peptide analogue was measured as the rate of glucose incorporation into muscle glycogen (µmol /g tissue/hr), and represented by the mean ± SEM from eight determinations.

Basal blood glucose determination

Overnight-fasted Zucker fatty(fa/fa) female rats were anaesthetized with sodium pentobarbitone (60 mg/kg body weight). After 45 min, basal blood glucose samples were taken from the tail vein, followed immediately by intraperitoneal (i.p.) injection of saline (control) or the peptide analogue (test, 10 µmol/kg body weight) in 0.4 ml of saline. Blood samples were taken at 15, 30, 60, 90, 120, 150 minutes after injection, and the blood glucose level in each sample was measured immediately by the glucose oxidase method, using a YSI Model-2300 STAT glucose analyzer (Yellow Spring, Ohio). Six animals for each group were used.

Intravenous insulin tolerance test (IVITT)

IVITTs (0.1 U insulin/kg body weight) were performed on overnight-fasted Wistar male rats as previously described (Lim *et al*, 1992). Blood samples were taken for glucose estimation at 15, 30, 45, 60 min after the commencement of the tests. Six animals in each group were used.

Statistical analysis

The Student's t-test was used to analyze the results. P values of < 0.05 were accepted as statistically significant.

Example 1 Aminosuccinimide Modification of hGH Peptides

α-aminosuccinimide derivatives of hGH peptides were prepared by a two-step approach, in which the aspartyl^{11} β-methyl ester of hGH peptides is subjected to subsequent displacement of the ester group by the neighbouring amide nitrogen of Asn^{12}, resulting in formation of an α-aminosuccinimide derivative.

hGH peptides with an α-aminosuccinimide(Asu) modification in the aspartyl residue were prepared by methyl esterification of the β-carboxylic group of Asp^{11}, followed by base-catalyzed de-esterification and ring closure according to the procedure of Stephenson *et al* (Stephenson and Clarke, 1989). Peptide (80 µmol) was first esterified by 30 ml of 0.08 N hydrochloric acid (HCl) in methanol at 20ºC overnight. Purified peptide ester (50 µmol) was incubated in 100 mL of 0.2 M sodium phosphate buffer (pH 7.4) at 20ºC or 37ºC. Asu formation was monitored with RP-HPLC using an analytical C18-column (4.6 mm x 25 cm, Vydac) at 214 nm. The reaction was terminated by adding diethyl ether and the Asu-peptides were purified by RP-HPLC. The peptides synthesised for this study are summarised in Table 1. Peptide 3 was subsequently designated compound ADD9903.

Table 1

Sequences of Synthetic Peptides

Peptide	Sequence	Peptide Amide Analogues
1	INSB(22-25)	Arg-Gly-Phe-Phe (SEQ ID NO. 1)
2	Cha^{25}-INSB(22-25)	Arg-Gly-Phe-Cha (SEQ ID NO. 2)
3	D-Ala^{23}-INSB(22-25)	Arg-D-Ala-Phe-Phe (SEQ ID NO. 3)
4	INSREC(86-89)	Arg-Leu-Phe-Phe (SEQ ID NO. 4)
5	hGH(8-13)	Arg-Leu-Phe-Asp-Asn-Ala (SEQ ID NO. 5)
6	Asu^{11}-hGH(8-13)	Arg-Leu-Phe-Asu-Asn-Ala (SEQ ID NO. 6)
7	Asu^{11}-hGH(6-13)	Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala (SEQ ID NO. 7)
8	Lys^{8},Asu^{11}-hGH(6-13)	Leu-Ser-Lys-Leu-Phe-Asu-Asn-Ala (SEQ ID NO. 8)
9	Gly^{8},Asu^{11}-hGH(6-13)	Leu-Ser-Gly-Leu-Phe-Asu-Asn-Ala (SEQ ID NO. 9)
10	Tyr^{10},Asu^{11}-hGH(6-13)	Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala (SEQ ID NO. 10)
11	Gly^{10},Asu^{11}-hGH(6-13)	Leu-Ser-Arg-Leu-Gly-Asu-Asn-Ala (SEQ ID NO. 11)

INSB: insulin B-chain

INSREC: α-subunit of the insulin receptor

hGH: human growth hormone

Asu: aminosuccinimide

Cha: β-cyclohexyl-L-alanine

Samples were analyzed by RP-HPLC using a linear gradient of acetonitrile from 0%-40% over 40 min. Peak areas beneath the identified peaks were regarded as the molar quantities.

For example, 55% conversion of the hGH(8-13) peptide to the α-aminosuccinimide form could be achieved in 2.5 hr at 20ºC, as shown in Table 2.

Table 2

Aminosuccinimide Modification of hGH(8-13) Peptide

Temperature (ºC)	20			37		
Time	Composition			Composition		
(min)	E	I	A	E	I	A
0	100	0	0	100	0	0
3	96	4	0	92	8	0
15	89	11	0	27	52	21
30	81	19	0	17	44	39
60	67	30	4	3	17	80
150	32	55	13	< 1	1	> 98
240	29	49	22	< 1	< 1	> 99

E = aspartyl^{11} β-methyl ester of hGH(8-13),
I = Asu^{11}-hGH(8-13),
A = hGH(8-13) and other isomers.

However, aspartyl isomers of hGH(8-13) peptide were formed when the reaction was carried out either for a longer period or at higher temperature, due to the decomposition of the succinimide structure. Total yields of Asu^{11}-hGH peptide analogues were 35%-50%, as calculated from the initial Rink resin loading.

Example 2 Insulin-Potentiating Effects on Glycogen Synthesis

To determine the insulin-potentiating effect of the peptide analogues, the rates of incorporation of glucose into muscle glycogen were measured. The rates of glycogen synthesis (μmol/g tissue/hr) were 0.52 ± 0.05, 0.60 ± 0.04, 1.27 ± 0.06 and 1.52 ± 0.07 in response to 0.33, 1, 3.33 and 10 mU/ml insulin respectively, as shown in Figure 1. This indicated that the stimulation of glycogen production was markedly accelerated when the amount of insulin was greater than 1 mU/ml.

The insulin-potentiating effect of the peptide analogues was then observed by studying their dose response curves for glucose incorporation into glycogen in the presence of 1 mU/ml exogenous insulin. The effects of peptides 1, 3, 4, 6, 7, 8 and 10 were evident at doses higher than 0.01 μmol/ml, and continued to increase with increasing peptide concentration to 1 μmol/ml, as shown in Figures 2A and 2B. The maximum stimulation for the rate of glycogen synthesis, up to 1.44 ± 0.04 (μmol/g tissue/hr), was observed in response to 10 μmol/ml of Arg-D-Ala-Phe-Phe amide (Peptide 3). However, insulin-potentiating activity was abolished if either the Arg or the Phe residue of INSB(22-25) was replaced by Gly or β-cyclohexyl-L-alanine (Cha) respective, and if hGH(8-13) did not have the Asn modification.

Example 3 Hypoglycaemic Effect of INSB(22-25), INSREC(86-89) and Asu^{11}-hGH(8-13) Peptides in Zucker Fatty (fa/fa) Rats

The insulin-potentiating effects of the peptide analogues were demonstrated using insulin-resistant Zucker fatty (fa/fa) rats. The reduction of basal blood glucose levels in animals by different peptide analogues administered intraperitoneally (i.p.)at a dose of 10 μmol/kg body weight was measured for over 150 min. The results are shown in Figure 3.

Peptides 1, 2, 3 and 6 showed significant hypoglycaemic effects ($p < 0.005$) during 60-90 min after administration, as compared with the control animals which were given an identical volume of saline. The potency of the peptide analogues decreased in the following order: Arg-D-Ala-Phe-Phe > Arg-Gly-Phe-Phe > Arg-Leu-Phe-Phe > Arg-Leu-Phe-Asu-Asn-Ala.

The Arg-Gly-Phe-Cha and hGH(8-13) peptide amide analogues showed no hypoglycaemic effect.

Example 4 Structure-Function Study of hGH Peptide Analogues

IVITTs were performed on normal male Wistar rats after a single intravenous (i.v.) injection of the hGH peptide analogues at a dose of 5 μmol/kg body weight. The insulin-potentiating effects of peptides 6, 7, 8 and 10 on decreasing blood glucose levels of treated animals became significant since 30 min after the commencement of the test. Bioactivity was retained when the Arg^{8} or Phe^{10} residue of Asu^{11}-hGH(6-13) peptide was substituted with Lys or Tyr respectively (1.92 ± 0.17 or 1.62 ± 0.18 vs. 1.65 ± 0.12 mmol/L at 45 min), as shown in Table 3. However, no insulin-potentiating effect was observed when either Arg^{8} or Phe^{10} was substituted by Gly (0.92 ± 0.08 or 1.08 ± 0.08 mmol/L respectively vs. 1.00 ± 0.10 mmol/L of control at 45 min). Asu^{11}-hGH(8-13) also elicited this insulin-potentiating effect, but with lower potency. Its linear analogue, Asn^{11}-hGH(8-13), showed no such effect.

Table 3

Potentiating Effect of hGH Peptide Analogues (5 µmol/kg body weight) on Intravenous Insulin Tolerance Tests (IVITTs).

Time (min)	15	30	45	60
Peptide	Reduction in Blood Glucose (mmol/L)			
5	0.90±0.09	1.01±0.11	1.07±0.15	1.06±0.12
6	0.93±0.17	1.58±0.12*	1.50±0.15*	1.50±0.19*
7	1.08±0.09	1.38±0.13*	1.65±0.12*	1.77±0.19*
8	1.08±0.10	1.35±0.14*	1.92±0.17*	1.78±0.17*
9	0.87±0.12	0.80±0.14	0.88±0.15	0.74±0.16
10	1.03±0.10	1.52±0.12*	1.62±0.18*	1.60±0.34*
11	0.68±0.05	0.96±0.06	1.08±0.08	1.12±0.06
Control (without peptide)	0.86±0.10	0.92±0.14	1.00±0.10	0.98±0.10

All data represent the Mean ± SEM for 6 animals in each group.

* denotes that differences between the peptide treated and control group are statistically significant($p<0.05$) at the indicated time. Basal blood glucose level of all animals was 3.4 ± 0.4 mmol/L before experimentation.

Example 5 Effect of acute oral administration

Overnight fasted Zucker fatty (*fa/fa*) female rats were administered peptide 3 (ADD9903) by oral gavage at a concentration of 20 µmol/kg of body weight. Rats were then immediately anaesthetized with nembutal administered intraperitoneally in order to avoid variations arising due to activity of the rats. Blood samples were collected

from the tail vein at time 0 min (immediately after oral gavage and before anaesthetic), 60 min, 120 min and 180 min, and analyzed for blood glucose by the glucose oxidation method using a YSI Model-2300 STAT glucose analyzer (Yellow Spring, Ohio). Six rats were analyzed for each of the control and treated groups.

The oral administration of peptide 3 to female Zucker (*fa/fa*) rats significantly reduced blood glucose levels compared to control rats. A significant reduction in blood glucose was observed 60 min after peptide 3 administration ($P < 0.05$) with a maximal decrease observed after 120 min ($P < 0.005$). Furthermore, oral administration of peptide 3 resulted in a more profound decrease in blood glucose at 120 min, compared to intraperitoneal administration of peptide 3 at the same time point ($P < 0.005$).

Example 6 Effect of chronic administration of peptide 3

The effects of chronic administration of peptide 3 in the C57Bl/6J *ob/ob* diabetic model were evaluated by measurements of a number of parameters, including body weight, food intake, plasma glucose levels, plasma insulin levels, intraperitoneal glucose tolerance test and glucose uptake by adipose tissue (*ex vivo*).

Male and female C57BL/6J *ob/ob* mice aged 12-15 weeks old were used. Fasting blood glucose levels were determined for all mice 14 days prior to experimentation. Only mice with fasting blood glucose levels >7.0mmol/l were used in the study.

Mice selected for this experiment were initially fasted for 4 hours, then anaesthetized with a single injection of sodium pentobarbitone (35mg/kg). A blood sample was collected from each mouse by eye-bleed for the assessment of plasma glucose and insulin levels (day 0). Collected blood samples were stored at -20°C until analysis was performed. A single Alzet mini-pump (#1002, Alzet, USA)

containing either sterile saline (100µl; n=14) or peptide 3 (20 µmol/kg dissolved in 100µl saline; n=14) was inserted under the skin between the scapula of the mice. The incision was clamped and disinfected using iodine. The pumps were left for 14 days, and 5 body-weight, and food measurements were recorded during this period, at days 0, 4, 7, 10 and 14. Blood samples were collected on day 0 and 14 days post-saline (n=5) or peptide 3 (n=4) administration for plasma glucose analysis. The results reported below are expresses as mean +/- standard error.

(a) Plasma insulin Plasma insulin levels were quantitatively determined in *ob/ob* mice, using an insulin radioimmunoassay kit (Linco Research Inc. USA) according to the manufacturer's instructions. Plasma insulin was quantitated for saline-treated (n=4) and peptide 3-treated mice (n=4).

(b) Intraperitoneal glucose tolerance test (IPGTT) An intraperitoneal glucose tolerance test was conducted to determine whether the clearance of a glucose load was enhanced. Ten mice were used in each group, five receiving saline and five receiving peptide 3. At 14 days after chronic administration of saline or peptide 3, mice were fasted for 4 hours, then anaesthetized and eye-bled for day 14 plasma metabolite analysis. Half of each saline or peptide 3 treatment group was given a single intraperitoneal injection of glucose (1g/kg dissolved in saline), and the other half saline (equivalent dose). Mice were eye-bled at 30, 60 and 120 minutes after glucose administration, and blood glucose levels were determined.

(c) Glucose transport assay Glucose transport in adipose tissue extracted from saline-treated and chronically peptide 3-treated mice was analyzed by an *ex vivo* glucose uptake assay. Adipose tissues was harvested from mice which had received saline (n=5) or peptide 3

(n=4) in the IPGTT, and used for a glucose transport assay. Mice were sacrificed by a lethal injection of pentabarbitone to the heart. Epididymal fat from male mice or peritoneal fat from female mice was used. Adipose tissues were rinsed in saline and then sliced into even pieces for weight determination. Tissues were placed in flasks and incubated in 2 ml KRB buffer (pH 7.4) containing D-glucose (10 mM final concentration) with vigorous agitation at 37°C for 2 h under an atmosphere of carbogen. All samples were then placed on ice to reduce glycolysis. Tissues were removed from flasks, and the remaining solutions were analyzed for glucose concentrations using a glucose analyzer. Glucose uptake by each tissue sample was calculated, and compared to tissue free buffer controls.

There was no significant difference in body weight gain or food intake between the saline-infused and peptide 3-infused mice over 14 days of treatment. However, plasma glucose levels were significantly decreased when mice were continuously infused with peptide 3 for 14 days, compared to saline-infused control mice ($P < 0.025$). Mice treated with peptide 3 exhibited a reduction of 11.60 $\pm$ 3.63 mmol/l in plasma glucose levels, compared to a negligible increase of 2.38 $\pm$ 1.81 mmol/l in saline-infused mice ($P < 0.005$), which is indicative of fasting (4h) plasma glucose measurements. These data suggest that chronic peptide 3 treatment significantly improved glucose clearance from the circulation.

The plasma insulin level observed in mice treated with peptide 3 for 14 days was significantly reduced compared to saline-treated mice (17.10 $\pm$ 5.99 ng/ml and 52.75 $\pm$ 10.10 ng/ml respectively; $P < 0.01$). This suggests that mice chronically treated with peptide 3 produce less insulin, as their blood glucose is being cleared more efficiently from the circulation and glucose transport into specific tissues such as adipose tissue is increased, as demonstrated in this study (see below).

Prior to glucose injection, peptide 3-treated

mice were demonstrated to have a lower basal blood glucose level of 46.1% compared to saline-treated mice ($P < 0.01$). The injection of a bolus of glucose into mice resulted in an increase in plasma glucose by 115% in peptide 3-treated and saline-treated mice respectively after 30 min. However, the level of blood glucose in peptide 3-treated mice was reduced by 47% at 120 min after glucose injection compared to saline-treated mice; this decrease was significant ($P < 0.03$). These results suggest that glucose is cleared more efficiently in mice chronically treated with peptide 3, and therefore a reduced hyperglycaemic effect is observed following glucose load.

Adipose tissue extracted from mice treated with peptide 3 for 14 days was shown to transport 38% more glucose (1.67 $\pm$ 0.18 nmol/mg tissue/h) than adipose tissue from saline-treated mice (1.22 + 0.18 nmol/mg tissue/h ($P < 0.05$). Thus chronic administration of peptide 3 results in enhanced glucose removal from the circulation to tissue, where it may be stored as fat or oxidized for energy utilization.

Example 7 Analysis of synthetic peptide analogues

In this example the peptide analogues were manually synthesized using solid-phase peptide synthesis by the Fmoc-strategy on a Rink amide acid, DIC (diisopropylcarbodiimide) and HOBt (1-hydroxybenzotriazol), using conditions slightly modified from those described above . Coupling was complete after incubation for 2 h. Fmoc was removed with piperidine/DMF. The final peptides were cleaved from the resin by treatment with trifluoroacetic acid, crystalline phenol, EDT and thioanisole. The filtrate from the cleavage reaction was precipitated in the ether solvent at 0°C. The precipitate was dissolved in acetonitrile/H_2O.

Peptides were purified by reversed-phase high performance liquid chromatography using a preparative C18

column and an acetonitrile gradient.

The activity of each analogue was assessed by *in vitro* measurement of glycogen synthesis in muscle, as described above.

The amino acids tested for each position in the tetrapeptide of general formula W-X-Y-Z as defined in the "Summary of the Invention" are set out in Table 4, and the activity results are summarized in Tables 5 and 6.

Table 4

W	X	Y	Z
Lysine	Glycine	Phenylalanine	Phenylalanine
Arginine	Leucine	Tyrosine	Tyrosine
Homolysine	Alanine		Tryptophan
Homoarginine	β-Alanine		Histidine
Ornithine	Isoleucine		Homophenylanine
	Cyclohexylalanine		

Table 5

Sequences of synthetic peptides and activity as measured in an *in vitro* glycogen synthesis assay in Zucker (*fa/fa*) rat hemidiaphragm muscle.

PEPTIDE	SEQUENCE	PEPTIDE AMIDE ANALOGUE	ACTIVITY
1	INSB (22-25)	Arg-Gly-Phe-Phe	insulin-potentiating *
2	Cha^{25}-INSB (22-25)	Arg-Gly-Phe-Cha	insulin antagonist
3	D-Ala23-INSB (22-25)	Arg-D-*Ala*-Phe-Phe	insulin-potentiating **
4	INSREC (86-89)	Arg-Leu-Phe-Phe	insulin-potentiating *
5	hGH (8-13)	Arg-Leu-Phe-Asp-Asn-Ala	inactive
6	Asu^{11}-hGH (8-13)	Arg-Leu-Phe-Asu-Asn-Ala	insulin-potentiating *
7	Asu^{11}-hGH (6-13)	Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala	insulin-potentiating *
8	Lys^{8}, Asu^{11}-hGH (6-13)	Leu-Ser-Lys-Leu-Phe-Asu-Asn-Ala	insulin-potentiating *
9	Gly^{8}, Asu^{11}-hGH (6-13)	Leu-Ser-Gly-Leu-Phe-Asu-Asn-Ala	inactive
10	Tyr^{10}, Asu^{11}-hGH (6-13)	Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala	insulin-potentiating *
11	Gly^{10}, Asu^{11}-hGH (6-13)	Leu-Ser-Arg-Leu-Gly-Asu-Asn-Ala	inactive

12	Ala^{22}-INSB (22-25)	Ala-Gly-Phe-Phe	inactive
13	Ala^{23}-INSB (22-25)	Arg-Ala-Phe-Phe	inactive
14	Ala^{24}-INSB (22-25)	Arg-Gly-Ala-Phe	inactive
15	Ala^{25}-INSB (22-25)	Arg-Gly-Phe-Ala	inactive
16	Lys^{22}-INSB (22-25)	Lys-Gly-Phe-Phe	inactive
17	Orn^{22}-INSB (22-25)	Orn-Gly-Phe-Phe	inactive
18	β-Ala^{23}-INSB (22-25)	Arg-β-Ala-Phe-Phe	insulin-potentiating *
19	Tyr^{24}-INSB (22-25)	Arg-Gly-Tyr-Phe	insulin-potentiating *
20	Cha^{24}-INSB (22-25)	Arg-Gly-Cha-Phe	insulin antagonist
21	Tyr^{25}-INSB (22-25)	Arg-Gly-Phe-Tyr	inactive
22	D-Ala^{23}-INSB (22-25)	Arg-D-Ala-Phe-Tyr	insulin-potentiating **
23	D-Ala^{23}, Tyr-me^{25}-INSB (22-25)	Arg-D-Ala-Phe-Tyr-me	insulin-potentiating **
24	D-Ala^{23}, Tyr^{24}-INSB (22-25)	Arg-D-Ala-Tyr-Phe	insulin-potentiating ***

INSB: insulin B-chain

INSREC: α-subunit of the insulin receptor

hGH: human growth hormone

Asu: aminosuccinimide

Cha: β-cyclohexyl-L-alanine

Orn: ornithine

All residues are of L-configuration unless indicated by "D".

Activity:

insulin-potentiating: improved activity compared to insulin alone
inactive: equal activity compared to insulin alone
insulin antagonist: reduced activity compared to insulin alone
*-** increasing insulin-potentiating activity

The following conclusions regarding the activity of the INSB tetrapeptides can be drawn from the results presented in Table 5, and are summarised in Table 6:

Position W: Arginine seems to be required for activity for the INSB tetrapeptides. When lysine (peptide 16) or ornithine (peptide 17) is substituted for arginine there is a loss of activity.

Position X: All possible substitutions have not yet been tested in this position. However, for glycine the activity seems to be determined by the amino acids that follow, ie. positions Y and Position Z: Alanine is inactive, but the D-alanine and β-alanine forms are active.

Position Y: Phenylalanine and tyrosine can be replaced, but activity is determined by the amino acid preceding this position ie. amino acid X.

Position Z: Only phenylalanine and tyrosine have been tested in this position. Again, activity is determined by the amino acid in position X.

However, the activity of longer peptides may be modulated by the N- or C-terminal extension; for example, peptide 8 is active, although it has lysine instead of arginine at position W.

The amino acid substitutions of the tetrapeptide allow the aromatic rings and side chains to maintain a conformation that allows high affinity binding to the target sequence.

Table 6 (corrected)

Peptide (SEQ ID NO)	Sequence	Activity
1	Arg-**Gly**-Phe-Phe-NH_2	active
2	Arg-**Gly**-Phe-Cha-NH_2	antagonist
14	Arg-**Gly**-Ala-Phe-NH_2	inactive
15	Arg-**Gly**-Phe-Ala-NH_2	inactive
19	Arg-**Gly**-Tyr-Phe-NH_2	active
20	Arg-**Gly**-Cha-Phe-NH_2	antagonist
21	Arg-**Gly**-Phe-Tyr-NH_2	inactive
4	Arg-**Leu**-Phe-Phe-NH_2	active
13	Arg-**Ala**-Phe-Phe-NH_2	inactive
3	Arg-**D-Ala**-Phe-Phe-NH_2	active
18	Arg-**β-Ala**-Phe-Phe-NH_2	active
22	Arg-**D-Ala**-Phe-Tyr-NH_2	active
23	Arg-**D-Ala**-Phe-Tyr-me-NH_2	active
24	Arg-**D-Ala**-Tyr-Phe-NH_2	active

DISCUSSION

The insulin-potentiating effect of INSB(22-25)-NH_2, a tetrapeptide amide, has been demonstrated in normal rats (Ng *et al*, 1989; Weitzel *et al*, 1971). The evidence indicated that the amino acid sequence is essential for hormonal function. The Arg^{B22} residue is important for bioactivity, since an Ala^{B22}-substituted analogue was found to be inactive (Weitzel *et al*, 1971). The guanidinium functional group of Arg frequently plays a crucial role in the biological activities of proteins and peptides (Hannon and Anslyn, 1993). Phe^{B24} and Phe^{B25} are two residues which are invariant and important in animal insulins during evolution, and are critical for receptor binding. Tager *et al* (1979) reported the discovery of a mutant insulin from a diabetic patient in which the phenylalanine at B24 or B25 is replaced by leucine, and showed that the activity of the mutant insulin was reduced almost one hundred fold. It has been suggested that the Phe^{B25} residue of the insulin molecule interacts with the Phe^{89} of the α-subunit of the insulin receptor molecule by means of an aromatic-aromatic interaction, resulting in hormone-receptor binding (Sabesan and Harper, 1980).

In the present study, the insulin-potentiating effects of peptide analogues derived from insulin, insulin receptor and hGH were examined both *in vitro* and *in vivo*. Peptide analogues were designed and synthesized in order to identify those residues responsible for bioactivity (Tables 2, 5 and 6). Our results indicated that the Arg-Gly-Phe-Phe (i.e. INSB(22-25)) amide peptide had insulin-potentiating effects; it stimulated glycogen synthesis in tissues *in vitro*, and reduced basal blood glucose levels *in vivo* in insulin-resistant Zucker fatty (fa/fa) rats. The findings with the INSB(22-25) peptide are consistent with our previous observation of a similar effect during IVITT in normal Wistar rats (Ng *et al*, 1989).

An increased insulin-potentiating effect was observed when Gly^{B23} was replaced by a D-Ala residue. In

particular, significantly increased ($p < 0.05$) on *in vitro* glycogen synthesis was observed in the presence of 0.01-1 μ mol/ml Arg-D-Ala-Phe-Phe amide (Figure 2A). Increased potency of the *in vivo* hypoglycaemic effect of this D-Ala substituted peptide analogue was also observed (Figure 3). This change is likely to prevent the degradation of D-Ala^{B23}-INSB(22-25) by the proteolytic attack of tissue enzymes, as is usually observed in peptides with D-amino acid substitutions (Zhang, 1989). INSREC(86-89) amide displayed similar but less striking effects both *in vitro* and *in vivo*.

In contrast, the bioactivity was lost when the Phe^{B25} residue was substituted by its saturated counterpart, β-cyclohexyl-L-alanine(Cha) (Armstrong *et al*, 1993 and Figures 2A & 3). Asu^{11}-hGH(8-13) amide, in which residues 8-11 are homologous to INSREC(86-89), showed a diminished insulin-potentiating effect (Figure 2A and Figure 3). The Asu^{11} group may mimic the molecular structure of the aromatic side-chain of the Phe^{B25} residue. However, the decrease in activity may result from facile hydrolytic opening of the α-aminosuccinimide ring at physiological temperature and pH (Table 2). Our evidence suggests that the residue at this position of the tetrapeptide motif should be of an unsaturated and cyclic structure to elicit the insulin-potentiating effect.

The insulin-potentiating effects of the peptides were further confirmed by results of intravenous insulin tolerance tests (IVITTs) with a series of hGH peptide analogues. Structure-activity relationships of peptide analogues revealed that the Arg^{8}, Phe^{10}and Asu^{11} residues are crucial for bioactivity. Replacement of Arg^{8} or Phe^{10} with Lys or Tyr respectively showed equivalent insulin-potentiating activity because of the structural similarity between Arg and Lys and between Phe and Tyr. The activity was dramatically reduced when residue 8 or 10 was substituted by Gly (Tables 3,5). Asu^{11}-hGH(8-13) peptide amide showed a similar but less potent bioactivity than

that of Asu^{11}-hGH(6-13) peptide amide (Tables 3,5). However, linear hGH(8-13) had no activity. Robson also showed that the bioactivity of hGH peptides was lost when the Asu residue was substituted by an acyclic amino acid such as Ala, Asp or Gly (Robson, 1986).

In summary, our results clearly indicate that the insulin-potentiating activity is characteristic of a molecular motif with sequence homology to amino acid residues 22-25 of the B-chain of insulin, residues 86-89 of the α-subunit of insulin receptor and residues 8-11 of hGH. This biological activity appears to be intrinsic to a four-residue motif with a basic amino acid in position 1, a neutral aliphatic amino acid in position 2, an aromatic amino acid in position 3, and an amino acid with a side-chain having π or non-binding electrons in position 4. The insulin-potentiating effect of Asu^{11}-hGH(6-13) peptide has been shown to be mediated by stimulating insulin receptor tyrosine kinase activity (Lim *et al*, 1994).

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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CLAIMS

1. A peptide or peptidomimetic compound which has the ability to potentiate one or more of the physiological activities of insulin, in which the peptide comprises the sequence:

W-X-Y-Z

in which W is a basic amino acid;

X is a neutral aliphatic amino acid, in either the L- or the D-form;

Y is an aromatic amino acid; and

Z is an amino acid or amino acid analogue which has a side chain having π or delocalised electrons,

with the proviso that where the compound is a peptide, it is not Arg-Gly-Phe-Phe, Arg-Gly-Ser-Arg-Leu-Phe-Phe-Asn-Tyr-Ala-Leu-Val, Arg-Leu-Phe-Asu-Asn-Ala, or Leu-Ser-Arg-Leu-Phe-Asu-Asn-Ala.

2. A peptide according to claim 1, in which

W is lysine, arginine, homolysine, homoarginine or ornithine;

X is the L- or the D-form of glycine, leucine, alanine, β-alanine, isoleucine, homoleucine, norleucine, homonorleucine, cyclohexylalanine, or homocyclohexylalanine; and/or

Y is phenylalanine or tyrosine.

3. A peptide according to claim 1 or claim 2, in which the amino acid or amino acid analogue Z is one with a cyclic side chain.

4. A peptide according to any one of claims 1 to 3, in which Z is phenylalanine, tyrosine, tryptophan, α-amino succinimide, homophenylalanine or histidine.

5. A peptide according to any one of claims 1 to 4, in which W is arginine.

6. A peptide according to any one of claims 1 to 5, in which X is glycine, D-alanine, or β-alanine.

7. A peptide according to any one of claims 1 to 5, in which Y is phenylalanine or tyrosine.

8. A peptide according to any one of claims 1 to 7, in which Z is phenylalanine, tyrosine, or methyl-tyrosine.

9. A peptide according to any one of claims 1 to 8, which is extended at either the N- or C-terminal.

10. A peptide according to claim 9, in which the N-terminal extension is leucine-serine.

11. A peptide according to claim 9, in which the C-terminal extension is asparagine-alanine.

12. A peptide according to any one of claims 1 to 11, selected from the group consisting of

Arg-D-Ala-Phe-Phe	(SEQ ID NO. 3),
Arg-Leu-Phe-Phe	(SEQ ID NO. 4),
Arg-Leu-Phe-Asu-Asn-Ala	(SEQ ID NO. 6),
Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala	(SEQ ID NO. 7),
Leu-Ser-Lys-Leu-Phe-Asu-Asn-Ala	(SEQ ID NO. 8),
Leu-Ser-Arg-Leu-Tyr-Asu-Asn-Ala	(SEQ ID NO. 10),
Arg-β-Ala-Phe-Phe	(SEQ ID NO. 18),
Arg-Gly-Tyr-Phe	(SEQ ID NO. 19),
Arg-D-Ala-Phe-Tyr	(SEQ ID NO. 22),
Arg-D-Ala-Phe-Tyr-me	(SEQ ID NO. 23), and
Arg-D-Ala-Tyr-Phe	(SEQ ID NO. 24).

13. A peptide according to claim 12, which is Arg-D-Ala-Phe-Phe (SEQ ID NO. 3) or Arg-D-Ala-Tyr-Phe (SEQ ID NO. 24).

14. A peptidomimetic compound according to claim 1, in which W is replaced by an analogue of arginine.

15. A peptidomimetic compound according to claim 1 or claim 14, in which

(a) one or more amino acids is replaced by its corresponding D-amino acid, or

(b) one or more peptide bonds is replaced by a structure more resistant to metabolic degradation.

16. A composition comprising a peptide according to any one of claims 1 to 13, or a peptidomimetic compound according to claim 14 or claim 15, together with a pharmaceutically-acceptable carrier.

17. A method of treatment of a pathological condition associated with insulin resistance, comprising the step of administering an effective amount of a peptide according to according to any one of claims 1 to 13, or a peptidomimetic compound according to claim 14 or claim 15, to a subject in need of such treatment.

18. A method according to claim 17, in which the condition is non-insulin dependent diabetes mellitus or obesity.

19. A method according to claim 17 or claim 18, in which the condition is non-insulin dependent diabetes mellitus.

20. A method according to any one of claims 17 to 19, in which the peptide or peptidomimetic compound is administered at a dose in the range 0.1 to 100 mg/kg body weight.

21. A method according to any one of claims 17 to 20, in which the peptide or peptidomimetic compound is administered orally or sublingually.

22. A method of treatment of a pathological condition associated with insulin resistance, comprising the step of administering an effective amount of a compound which mimics the action of the binding region INSB 22:25 on the insulin receptor.

23. A method according to claim 23, in which the condition is non-insulin dependent diabetes mellitus.

24. Use of a peptide according to according to any one of claims 1 to 13, or a peptidomimetic compound according to claim 14 or claim 15, for the manufacture of a medicament for the treatment of a pathological condition associated with insulin resistance.

25. Use according to claim 23, in which the condition is non-insulin dependent diabetes mellitus or obesity.

26. Use according to claim 23 or claim 24, in which the condition is non-insulin dependent diabetes mellitus.

27. Use according to any one of claims 23 to 25, in which the peptide or peptidomimetic compound is administered at a dose in the range 0.1 to 100 mg/kg body weight.

28. Use according to any one of claims 23 to 26, in which the peptide or peptidomimetic compound is administered orally or sublingually.

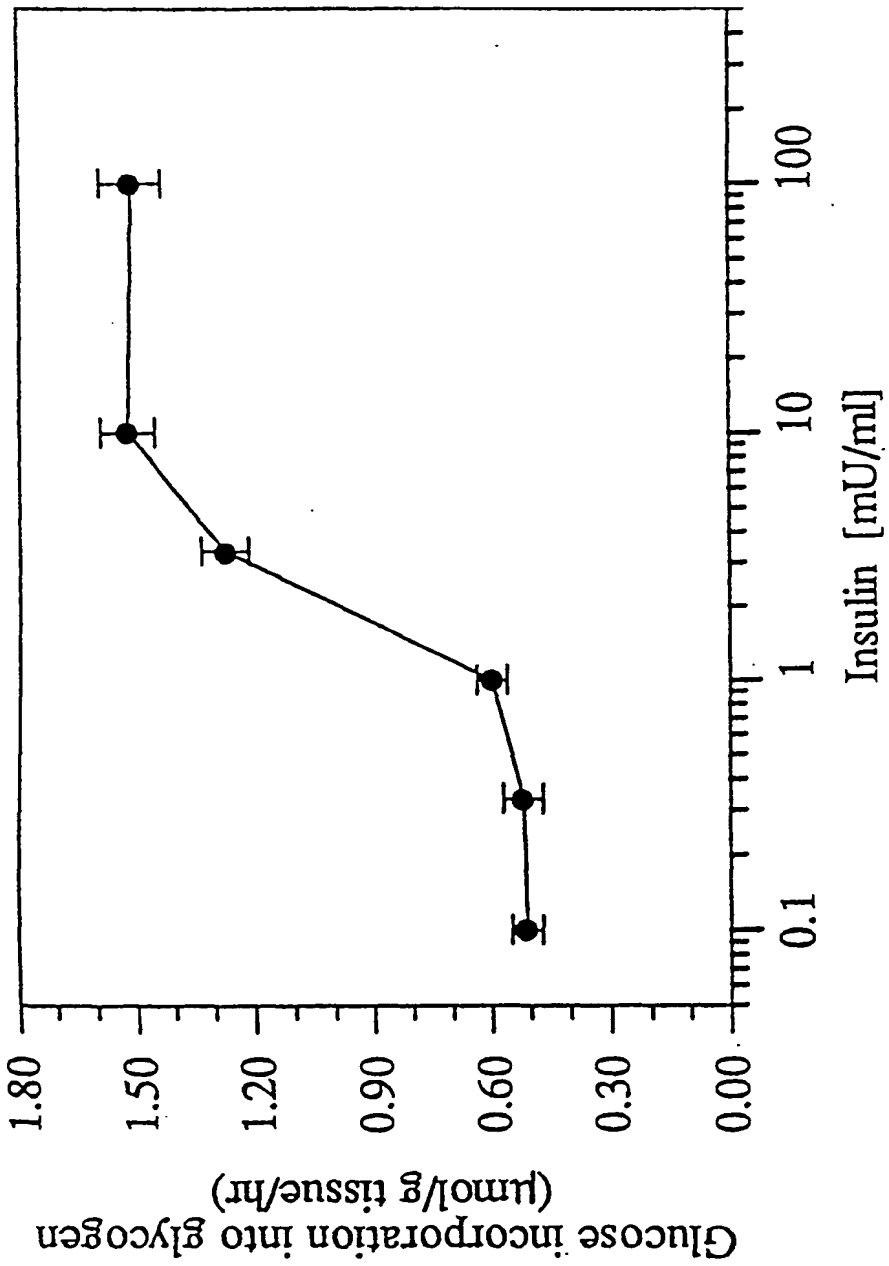
Glucose incorporation into glycogen
(μmol/g tissue/hr)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
0.1
1
10
100
Insulin [mU/ml]

FIGURE 1

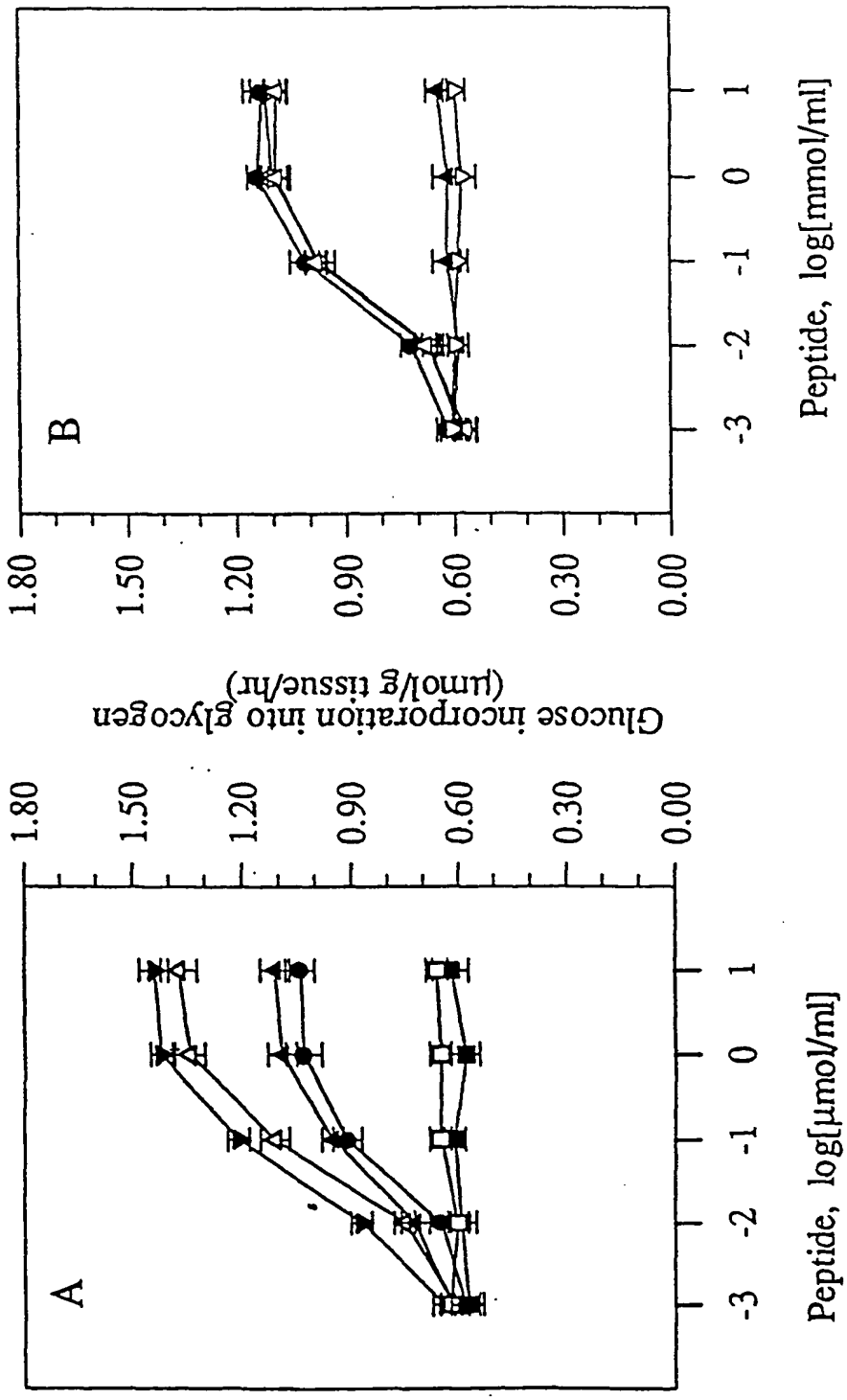
A
B
Glucose incorporation into glycogen
(μmol/g tissue/hr)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
-3
-2
-1
0
1
Peptide, log[μmol/ml]
Peptide, log[mmol/ml]

FIGURE 2

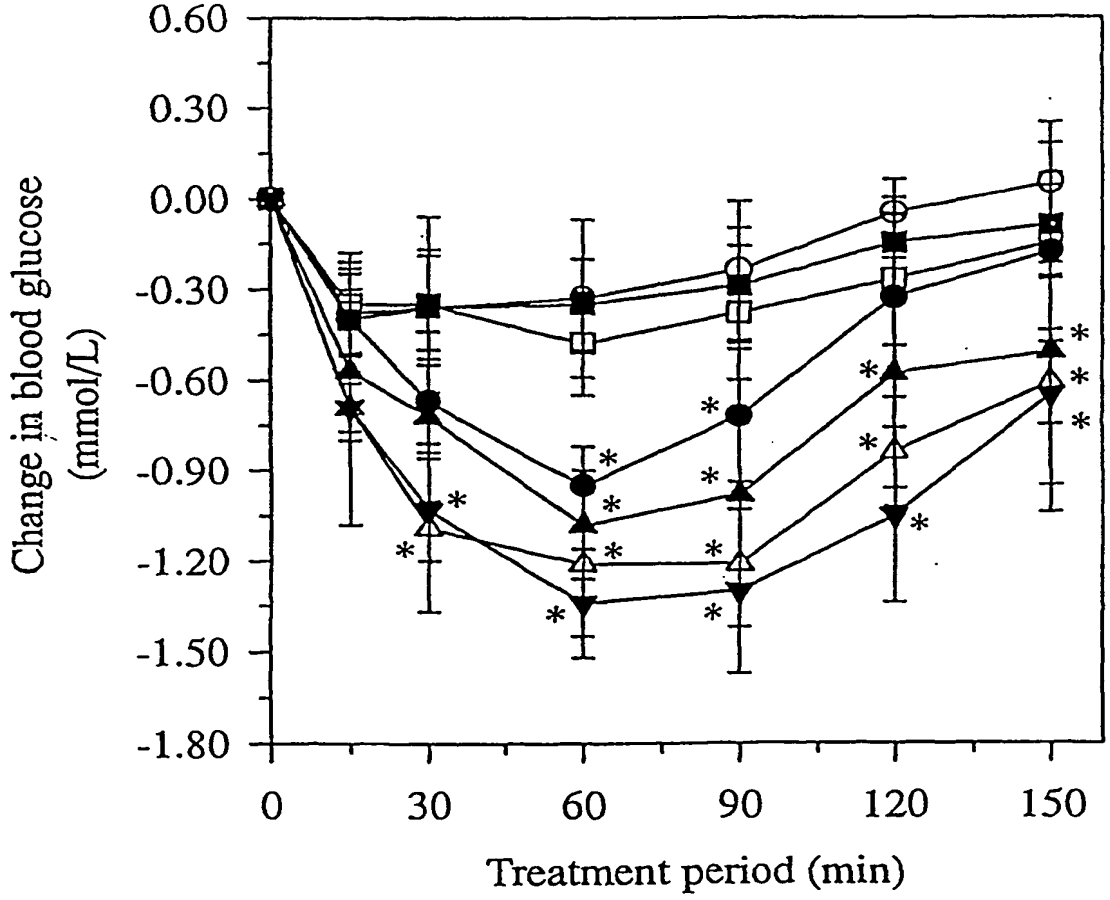
0.60
0.30
0.00
-0.30
-0.60
-0.90
-1.20
-1.50
-1.80
Change in blood glucose (mmol/L)
0
30
60
90
120
150
Treatment period (min)

FIGURE 3

SEQUENCE LISTING

<110> METABOLIC PHARMACEUTICALS LIMITED

<120> INSULIN-POTENTIATING COMPOUNDS

<130> FP14266 Metabolic Pharmaceuticals

<140> PCT/AU01/
<141> 2001-03-30

<150> PQ6618
<151> 2000-03-31

<160> 24

<170> PatentIn Ver. 2.1

<210> 1
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
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<223> AMIDATION

<220>
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<400> 1
Arg Gly Phe Phe
1

<210> 2
<211> 4
<212> PRT
<213> Artificial Sequence

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<222> (4)
<223> residue is Cyclohexylalanine

<220>
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<222> (4)
<223> AMIDATION

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(22-25)

<400> 2
Arg Gly Phe Xaa
1

<210> 3
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
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<222> (2)
<223> residue is D-Ala

<220>
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<223> AMIDATION

<220>
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(22-25)

<400> 3
Arg Xaa Phe Phe
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<210> 4
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
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<222> (4)
<223> AMIDATION

<220>
<223> Description of Artificial Sequence:INSREC (86-89)

<400> 4
Arg Leu Phe Phe
1

<210> 5
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> MOD_RES
<222> (6)
<223> AMIDATION

<220>
<223> Description of Artificial Sequence:hGH (8-13)

<400> 5
Arg Leu Phe Asp Asn Ala
1 5

<210> 6
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
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<223> residue is aminosuccinimide

<220>
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<222> (6)
<223> AMIDATION

<220>
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(8-13)

<400> 6
Arg Leu Phe Xaa Asn Ala
1 5

<210> 7
<211> 8
<212> PRT
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<220>
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<220>
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<222> (8)
<223> AMIDATION

<220>
<223> Description of Artificial Sequence:Asu[11]-hGH (6-13)

<400> 7
Leu Ser Arg Leu Phe Xaa Asn Ala
1 5

<210> 8
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<223> residue is aminosuccinimide

<220>
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<400> 8
Leu Ser Lys Leu Phe Xaa Asn Ala
1 5

<210> 9
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<212> PRT
<213> Artificial Sequence

<220>
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<223> residue is aminosuccinimide

<220>
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<222> (8)
<223> AMIDATION

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Sequence:Gly[8],Asu[11]-hGH (6-13)

<400> 9
Leu Ser Gly Leu Phe Xaa Asn Ala
1 5

<210> 10
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Sequence:Tyr[10],Asu[11]-hGH (6-13)

<400> 10
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1 5

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Sequence:Gly[10],Asu[11]-hGH (6-13)

<400> 11
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<210> 12
<211> 4
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<220>
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(22-25)

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<210> 13
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(22-25)

<400> 13
Arg Ala Phe Phe
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<210> 14
<211> 4
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<220>
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(22-25)

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<210> 15
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(22-25)

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Arg Gly Phe Ala
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<211> 4
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<220>
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<220>
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<400> 16
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1

<210> 17
<211> 4
<212> PRT
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<220>
<221> MOD_RES
<222> (1)
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<220>
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<222> (4)
<223> AMIDATION

<220>
<223> Description of Artificial Sequence:Orn[22]-INSB (22-25)

<400> 17
Xaa Gly Phe Phe
1

<210> 18

<211> 4
<212> PRT
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<220>
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<223> residue is beta-Alanine

<220>
<221> MOD_RES
<222> (4)
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<220>
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Sequence:beta-Ala[23]-INSB (22-25)

<400> 18
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<210> 19
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<223> Description of Artificial Sequence:Tyr[24]-INSB
(22-25)

<400> 19
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<210> 20
<211> 4
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<221> SITE
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<220>
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<220>
<223> Description of Artificial Sequence:Cha[24]-INSB (22-25)

<400> 20
Arg Gly Xaa Phe
1

<210> 21
<211> 4
<212> PRT
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<220>
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<220>
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<210> 22
<211> 4
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<220>
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<220>

<221> MOD_RES
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<400> 22
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1

<210> 23
<211> 4
<212> PRT
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1

<210> 24
<211> 4
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<220>

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<220>
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<223> AMIDATION

<220>
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Tyr[24]-INSB (22-25)

<400> 24
Arg Xaa Tyr Phe
1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00354

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 5/10, 7/04, A61K 38/07, 38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WPIDS, CHEMICAL ABSTRACTS

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AS BELOW.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, Chemical Abstracts, SEQ. ID. Nos, 3,4,6,7,8,10,18,19,22,23,24

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kundu, B., et.al. Indian Journal of Biochemistry & Biophysics (1987) 24:344-7 Synthesis and Hypoglycemic Activity of Peptides Related to Insulin Fragments B22-25, (see Table 1)	1-28
X	GB, A, 1499764 (TAKEDA CHEMICAL INDUSTRIES LTD) 1 February 1978 (see p.1 line 25-27, and particularly example 9)	1-9,17-28
X	WO, A, 89/04323 (MONASH UNIVERSITY and AUSTRALASIAN DRUG DEVELOPMENT LIMITED) 18 May 1989 (see particularly Table 5, and p. 18 line 15-p. 19 line 11)	1-5,7,9-11,14-28

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2001	22 MAY 01
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	ALISTAIR BESTOW Telephone No : (02) 6283 2450

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00354

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pullin, C.O., *et.al.* International Journal of Peptide and Protein Research -Abstract. (PMID:7042618), (1981) 18(3):318-23 Insulin-potentiating action of human growth hormone. Synthesis and activity of N-terminal fragments.	1-5,7,9,11, 14-28
X	Lee, T., *et.al.* Journal of Peptide Research (1997) 49:394-403 Conformational stability of a type II' β-turn motif in human growth hormone [6-13] peptide analogues at hydrophobic surfaces.(see particularly Table 1, compound 1, and p. 394)	1-5,7,9-11, 14-28
X	Thompson, P.E., *et.al.* Drug Design and Discovery (1995) 13:55-72 Structure and *in vivo* Activity of Hypoglycaemic Analogues of Human Growth Hormone(6-13) (see Table 1, and p. 63 line 34 - p. 67 line 16)	1-5,7,9-11, 14-28
X	Ng, F.M., *et.al.* Diabetes (1980) 29:782-787 The Minimal Amino Acid Sequence of the Insulin-potentiating Fragments of Human Growth Hormone (see Table 1)	1-3,5,7,9-11, 14-28

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/00354

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
GB	1499764	AT	2239/75	AU	79046/75	BE	827160
		CA	1059993	CH	618959	DE	2513057
		DK	1262/75	ES	435990	FI	750925
		FR	2274604	JP	50129528	NL	7503519
		NO	751044	PH	13025	SE	7503471
		US	4001199	US	4073890	ZA	7501789
		JP	51086440				
WO	8904323	EP	386044	NZ	226764	US	6048840
		AU	26010/88				

END OF ANNEX